Validation of a New Kit for the Quantitative Determination of Chromogenic Factor IX Activity

Abstract: PB0100

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Background

Factor IX activity assay methodology falls into two categories: one-stage clot-based (OSC) assays using an activated partial thromboplastin time (APTT) and two-stage chromogenic substrate (CS) assays. Unlike APTT-based assays, the CS assay offers the advantage of being less prone to impact from common interferents in samples.

Aim

The aim of the study was to characterize a new chromogenic assay for the quantitative determination of factor IX (FIX) activity in citrated plasma samples by evaluating the reproducibility, reference interval, and on-board stability of the reagents.

Methods

All studies were performed according to applicable CLSI guidelines on IL ACL TOP series instruments.

Reproducibility was determined through a 5 day x 2 run x 3 replicate study using three reagent lots at a single site by testing six plasma samples containing FIX levels spanning the assay range.

The reference interval was measured by testing 120 frozen plasma samples (venipuncture, 3.2% citrate) from ostensibly healthy individuals with three lots of reagents on two analyzers and calculated using a non-parametric method (2.5^{th} to 97.5^{th} percentile).

On-board stability was assessed by repeated testing of five freshly-thawed plasma samples using the same set of reagents over the course of 49 hours.

Results

The between-lot and within-site assay precision was <10% CV for four plasma samples with high FIX (≥10%) activity and SD of <0.8% FIX for two low FIX (<10%) plasma samples **(Figure 1 and Table 1)**.

The assay reference interval was 74 to 151% FIX activity **(Table 2)**.

Reagents demonstrated a robust on-board stability over nine hours with highly consistent results across the time course **(Figure 2)**.

Conclusions

We observed excellent performance and lot-to-lot consistency of our factor IX chromogenic assay when measuring reproducibility, reference interval, and on-board stability. The reagents are stable throughout a typical working day and the convenient frozen format and packaging size expedites preparation time and minimizes reagent wastage.

Figure 1 – Reproducibility Measurements

The mean measured value (solid black line) and plus/minus two standard deviations (dashed lines) are shown.

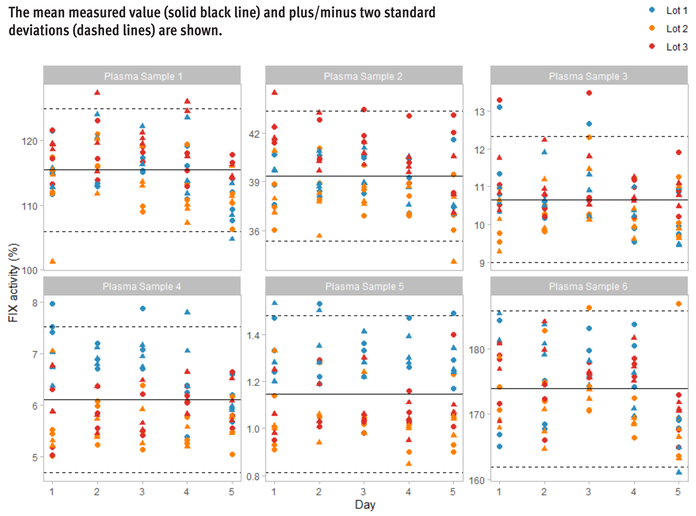

Table 1 – Precision Estimates

Plasma Sample	Mean FIX (%)	Repeatability		Between-Run		Between-Day		Between-Lot		Within-Site	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	115.44	3.91	3.39	0.00	0.00	2.03	1.76	2.53	2.19	5.08	4.40
2	39.35	1.37	3.49	0.56	1.42	0.42	1.07	1.58	4.01	2.21	5.61
3	10.66	0.74	6.95	0.19	1.81	0.17	1.63	0.34	3.23	0.86	8.05
4	1.15	0.11	NA	0.00	NA	0.00	NA	0.15	NA	0.19	NA
5	6.10	0.53	NA	0.00	NA	0.09	NA	0.59	NA	0.79	NA
6	173.86	5.34	3.07	0.69	0.4	2.53	1.46	1.49	0.86	6.14	3.53

Table 2 – Reference Interval

Reference Interval	Lower Limit 90% CI	Upper Limit 90% CI
74.48–150.71	69.08–81.52	144.94–156.69

Figure 2 – On Board Stability